# REPORT DOCUMENTATION PAGE

| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 30 November 2007 | | |

**4. TITLE AND SUBTITLE**

Relationship between growth hormone in vivo bioactivity, the insulin-like growth factor-I system and bone mineral density in young, physically fit men and women

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

B.C. Nindl, J.R. Pierce, M.J. Durkot, A.P. Tuckow, M.J. Kennett, J.W. Nieves, F. Cosman, J.A. Alemany, W.C. Hymer

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Military Performance Division
U.S. Army Research Institute of Environmental Medicine
42 Kansas Street
Natick, MA 01760-5007

**8. PERFORMING ORGANIZATION REPORT NUMBER**

M08-02

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick
Frederick, MD 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION/AVAILABILITY STATEMENT**

Approved for Public Release; unlimited distribution

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Context: Bone mineral density (BMD) is influenced by growth factors, such as growth hormone (GH) and insulin-like growth factor-I (IGF-I). The in vivo bioassay for GH (bioGH) provides a more physiologically relevant measurement than an in vitro immunoassay, since bioGH is quantified on a biological outcome. Objective: To determine if bioGH and components of the IGF-I system were associated with BMD in age matched men (M;n=41, $19.1 \pm 0.2$ year, $70 \pm 3$ kg, $163 \pm 25$ cm) and women (W;n=39, $18.6 \pm 0.3$ year, $66 \pm 3$ kg, $141 \pm 15$ cm). Design: Blood was analyzed for growth-related hormones (bioGH) immunoreactive growth hormone (iGH), IGF-I and associated binding proteins], and BMD was measured by pDXA, pQCT, and central DXA (spine, hip). For the bioGH assay, hypophysectomized female Sprague-Dawley rats were injected with a s.c. bolus of either a GH standard or unknown (each subject's plasma) in four daily injections. The tibia was then examined for epiphyseal growth plate width from which bioGH concentrations were extrapolated. Results: M had greater ($P<0.05$) calcaneal BMD when measured by pDXA (M: 1.27
$+ 0.02$; W; 1.14 $+ 0.02$ g/cm2) while pQCT assessed BMD at the tibia was not different (M: 777 $+$ 16; W; 799 $+$ 16 g/cm2). bioGH

**15. SUBJECT TERMS**

Bioassay, Hormones; Insulin-like growth factor-I binding proteins; pQCT

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | |
| unclassified | unclassified | unclassified | UL | 7 | 19b. TELEPHONE NUMBER *(Include area code)* |

# Relationship between growth hormone *in vivo* bioactivity, the insulin-like growth factor-I system and bone mineral density in young, physically fit men and women

B.C. Nindl [a,*], J.R. Pierce [a], M.J. Durkot [a], A.P. Tuckow [a], M.J. Kennett [b], J.W. Nieves [c], F. Cosman [c], J.A. Alemany [a], W.C. Hymer [b]

[a] *Military Performance Division, US Army Research Institute of Environmental Medicine, Kansas Street, Building 42, Natick, MA 01760-5007, USA*
[b] *Central Biological Laboratory, The Pennsylvania State University, State College, PA 01830, USA*
[c] *Clinical Research and Regional Bone Centers, Helen Hayes Hospital, West Haverstraw, NY, USA*

## Abstract

*Context:* Bone mineral density (BMD) is influenced by growth factors, such as growth hormone (GH) and insulin-like growth factor-I (IGF-I). The *in vivo* bioassay for GH (bioGH) provides a more physiologically relevant measurement than an *in vitro* immunoassay, since bioGH is quantified on a biological outcome.

*Objective:* To determine if bioGH and components of the IGF-I system were associated with BMD in age-matched men (M; $n = 41$, $19.1 \pm 0.2$ year, $70 \pm 3$ kg, $163 \pm 25$ cm) and women (W; $n = 39$, $18.6 \pm 0.3$ year, $66 \pm 3$ kg, $141 \pm 15$ cm).

*Design:* Blood was analyzed for growth-related hormones [bioGH, immunoreactive growth hormone (iGH), IGF-I and associated binding proteins], and BMD was measured by pDXA, pQCT, and central DXA (spine, hip). For the bioGH assay, hypophysectomized female Sprague–Dawley rats were injected with a s.c. bolus of either a GH standard or unknown (each subject's plasma) in four daily injections. The tibia was then examined for epiphyseal growth plate width from which bioGH concentrations were extrapolated.

*Results:* M had greater ($P < 0.05$) calcaneal BMD when measured by pDXA (M: $1.27 \pm 0.02$; W: $1.14 \pm 0.02$ g/cm$^2$), while pQCT-assessed BMD at the tibia was not different (M: $777 \pm 16$; W: $799 \pm 16$ g/cm$^2$). bioGH was similar between M ($5388 \pm 800$ µg/L) and W ($4282 \pm 643$ µg/L) and was not correlated with BMD. The only BMD-related biomarkers in women were acid-labile subunit (ALS; $r = 0.40$) and IGFBP-3 ($r = 0.42$) with DXA-measured spine and femoral neck BMD, and ALS ($r = 0.47$) with pQCT-assessed tibial BMD and cortical thickness, respectively.

*Conclusion:* Although bioGH was not associated with BMD, IGF-I and associated binding proteins (IGFBP-3 and ALS) emerged as correlates in W only.
Published by Elsevier Ltd.

*Keywords:* Bioassay; Hormones; Insulin-like growth factor-I binding proteins; pQCT

## 1. Introduction

The growth hormone/insulin-like growth factor-I (GH/IGF-I) axis plays a critical role in bone formation.

GH secreted from the anterior pituitary gland and its pulsatile secretion is known to be enhanced during longitudinal bone growth, and dampened during osteoporosis [1–4]. While many of the effects of GH are thought to be mediated indirectly *via* the IGF-I system, there is supporting evidence that GH also exerts direct effects on bone remodeling. In young animals in which the epiphyseal growth plate has not yet fused in long

bones, growth is inhibited by hypophysectomy (removal of the pituitary gland) and stimulated by GH administration [2, 5]. With GH administration, chrondrogenesis is accelerated, and as cartilaginous epiphyseal plates widen, more bone matrix is deposited at the ends of long bones. The molecular mechanisms underlying the GH physiological effects on bone remodeling appear to involve a potentiation of the signal transducers and activators of transcription (STATs), extracellular signal-regulated kinases (ERKs) and mitogen-activated protein kinases (MAPKs), which are all involved in the in the regulation of osteoblastic cell growth and/or differentiation [6, 7]. For example, using the human osteoblastic cell line Saos-2, Ziros et al. [6], demonstrated that GH signaling through Stat3/ERK MAPK potentiated the DNA binding activity of Runx2 (one of the master regulators of osteoblast differentiation).

There are many biochemical methods with which to measure GH. For instance, an increase in the width of the tibial epiphyseal plate in hypophysectomized rats has been used as an *in vivo* bioassay for GH ("tibia test"). GH concentrations obtained in this manner are termed bioassayable growth hormone (bioGH). The "tibia test" offers the most accurate measurement of biologically active GH and thus has advantages over traditional radioimmunoassays (RIAs), which measure immunodetectability between an antibody and an epitope on a molecule. The tibial bioassay offers insight into the actual intrinsic "biological activity" of GH, whereas immunoassays only yield information about the "immunodetectability" of human growth hormone based upon the interaction of an antibody with an epitope on the protein of interest. bioGH obtained from the tibia test has been considered a bonafide *in vivo* bioassay for GH, since its first report in 1949 [2]. Since then, work has firmly established that a dichotomy exists between immunoreactive (iGH) and bioactive GH [8–10]; yet no studies to date have examined whether bioGH is associated with actual *in vivo* measures of bone health in men and women.

It is well known that high bone content lowers skeletal injury risk; therefore, screening methods targeted at identifying individuals predisposed to skeletal pathologies (i.e., stress fractures and later osteoporosis) may minimize the chance of a future injury through early interventions. Based on the relationship that bioGH shares with tibial plate growth [2], it is possible that the level of bioGH would parallel the amount of bone mineral density (BMD) present. In a previous report that did not directly measure GH, we observed that IGF-I and IGFBP-3 (both under the direct stimulation of GH) were associated with DXA-assessed bone mineral content in women, but not in men [11]. The current study sought to broaden our understanding of the relationship between endogenous circulating components of the GH/IGF-I axis and bone health in men and women.

This study employed the tibial GH bioassay for the purpose of assessing bioGH in young, physically fit men and women, and to determine whether bioGH correlated with dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) estimates of bone mineral density. The current study tested the hypothesis that *in vivo* growth hormone bioactivity (bioGH) and downstream mediators of GH, such as IGF-I and its associated binding proteins, would be associated with *in vivo* DXA and pQCT measures of bone health in men and women.

## 2. Methods

### 2.1. Subjects

Male (M; $n = 41$, $19.1 \pm 0.2$ year, $70 \pm 3$ kg, $163 \pm 25$ cm) and female (W; $n = 39$, $18.6 \pm 0.3$ year, $66 \pm 3$ kg, $141 \pm 15$ cm) cadets from the US Military Academy (USMA) volunteered for this study. All volunteers provided written informed consent approved by the appropriate scientific and human use review committees at the US Army Research Institute of Environmental Medicine (USARIEM) and the USMA. The volunteers were from the USMA Class of 2002, and drawn from a larger cohort of cadets participating in a longitudinal study on bone health at the USMA [12, 13]. A pre-participation health examination revealed that subjects were free from any endocrine disorders known to influence bone health. All data collection was accomplished during one visit: DXA scan, peripheral DXA (pDXA) scan, pQCT scan, and blood draw.

### 2.2. Bone densitometry measures

BMD ($g/cm^2$) of the left calcaneus was measured by DXA (pDXA; Pixi, Lunar, Madison, WI) in all cadets. Peripheral quantitative computed tomography (pQCT; Stratec, Germany XCT-2000) was used to image a single slice at the two-third distal tibia. The distal third of the tibia was determined by a manual measurement of tibial length between the base of the patella and the lateral malleolus of the fibula to the closest centimeter. The 2/3 site was then located by the pQCT scanner after placing a positioning light of the gantry above the styloid process. Bone mineral content (mg per 1 mm slice of bone), bone density ($mg/cm^3$), cortical thickness (mm) and periosteal circumference (mm) were measured. Cortical thickness was derived using the circular ring model, using a threshold of $710 \, mg/cm^3$ to define cortical bone. This model calculates a mean cortical thickness from measures of total bone area and cortical bone area. Spine (lumbar vertebral bodies: L2–L4), and hip (total hip and femoral neck) BMD was measured using standardized positioning devices and the high-resolution

software mode in a mobile DXA scanner (DPX-IQ, Lunar, Madison WI). The coefficient of variation for each bone measurement was calculated by scanning 10 individuals on each machine twice. The coefficient of variation for bone density *in vivo* was 1.0%, 1.2%, 1.5% and 1.5% for the calcaneus by pDXA, tibia by pQCT, spine and hip by DXA, respectively.

### 2.3. Hormonal analyses

Blood was obtained *via* venipuncture in the morning (between 0700 and 1200). Subjects were instructed not to exercise or engage in strenuous physical activity before reporting to the laboratory. After collection, tubes were inverted and gently shaken to prevent coagulation, then centrifuged for 30 min at 800*g* at 4 °C. After centrifugation, plasma was aliquoted into a preservative tube, flash frozen in liquid nitrogen, and stored at −80 °C until later analysis.

The IGF-I system assays including total and free IGF-I, and IGFBP-3, as well as iGH were analyzed using commercially available IRMA assays (DSL; Diagnostic Systems Laboratories, Webster, TX). Two additional IGF-I related binding proteins, ALS and IGFBP-2, were analyzed using a commercially available ELISA and RIA assays, respectively (DSL, Webster, TX). The bone biomarkers, osteocalcin and intact parathyroid hormone (iPTH), were measured using commercially available IRMA and ELISA assays, respectively (DSL, Webster, TX). Serum C-telopeptides of type I collagen (CTX) was quantified using an electrochemiluminescence immunoassay (Nordic Bioscience Diagnostics, Herlev, Denmark), and serum cross-linked N-teleopeptide of type I collagen (NTx) was measured using the Osteomark ELISA (Inverness Medical, Princeton, NJ).

The IRMA assays were read on a Cobra gamma counter (Packard Instruments, Downers Grove, IL), and the ELISA kits were read on a Dynex MRX Revelation absorbance reader (Dynex Technologies, Chantilly, VA). All samples were ran in duplicate, and inter- and intra-assay CV's for all of the above ELISA kits were <10%. Within the IRMA and electrochemiluminescence assays, analyte measurements were assayed in the same batch to eliminate inter-assay variance, while intra-assay variance was below 10%.

### 2.4. Determination of growth hormone in vivo bioactivity

Concentrations of biologically active GH (bioGH) samples were determined according to the method of Greenspan et al. [2]. Female Sprague–Dawley rats (Hilltop Labs, Scottsdale, PA), hypophysectomized at 26–28 days of age, were used 2 week after surgery. All animals were housed in individual cages at an animal care facility at Penn State University, and all (*n* = 244) were treated following the guidelines for animal care in accordance with the USARIEM and The Pennsylvania State University Institutional Animal Care and Use Committees. Throughout the duration of the study, the animals were kept on a 12 h light–dark cycle, and were allowed to consume food and water *ad libitum*. Animals that weighed <80 g or >100 g at the time of sample injection were excluded. The following criteria were taken as evidence for completeness of hypophysectomy: failure to gain >7 g in the 10 days after the operation, deterioration of body tonus, maintenance of infantile ("smooth") hair, and absence of pituitary remnants in the sella turcica at autopsy as determined by visual inspection under magnification.

Subsequently, each of the experimental animals was injected with an s.c. bolus of the unknown sample or standard in four daily injections (over 4 consecutive days). Animals used for the development of the standard curve were injected with a total of 0, 10, 30 or 90 μg of hGH in 4 ml of 1.0 mM $(NH_4)$ $HCO_3$ buffer. Twenty-five hours after the last injection, the animals were euthanized by $CO_2$ asphyxiation. Both tibias were surgically removed, split longitudinally and stained with silver nitrate and plate widths were measured in double-blind fashion by two investigators whose readings concurred within 10%. Ten readings measured with an ocular micrometer were averaged across the plate width for each sample. GH responses were expressed in terms of a purified human pituitary preparation (3.0 IU/mg). A total of 25 different epiphyseal plates were used to generate the standard curve ($r^2 = 0.89$), and intra-assay CV was 7.2%.

### 2.5. Statistical analysis

All statistical analyses were performed using Statistica (Version 7.0). Values are reported as mean ± SEM, and the alpha level was set at $P \leqslant 0.05$ for all statistical tests. Descriptive statistics were calculated for all measures. Homogeneity of variance and normality of distribution were determined on all data by examining the skewness and kurtosis values (−1 to 1 was considered normal). All gender comparisons were performed with an independent Student's *t*-test. Additionally, to control for differences in body mass between men and women, analysis of covariance (ANCOVA) was used to assess gender differences in bone densitometry measures in which total tissue mass was used as a covariate. This procedure involved adjusting the overall means for the bone health variables based on differences in total mass between men and women. Levene's test of homogeneity of variance was confirmed ($P \leqslant 0.05$) indicating equal variances for the dependent bone measures in men and women, as was the linearity between the regional tissue mass of interest and total tissue mass. Pearson product-moment correlation coefficients (*r*) evaluated the relationship between bone densitometry (DXA or pQCT) and hormonal measures.

## 3. Results

### 3.1. Bone measures

Table 1 displays the absolute and adjusted mean values for all bone related variables for both men and women. Men had greater absolute values ($P \leqslant 0.05$) than women for all measures, with the exception of DXA-assessed spine BMD ($1.26 \pm 0.02$ g/cm$^2$ for both sexes) and pQCT-assessed tibial BMD ($798.7 \pm 16.1$ vs. $777.3 \pm 15.6$ mg/cm$^3$ for women and men, respectively). When adjusted for differences in body mass *via* the ANCOVA, women had a greater DXA-assessed spine BMD ($1.31 \pm 0.02$ vs. $1.22 \pm 0.02$ g/cm$^2$; $P \leqslant 0.05$) and similar values for pQCT-assessed cortical thickness (Females: $3.9 \pm 0.9$ vs. Males: $4.3 \pm 0.1$ mg/cm$^3$).

### 3.2. GH and IGF-I system

Table 2 displays the mean values of the GH/IGF-I system for both men and women. bioGH was similar between women and men, ($4282 \pm 643$ vs. $5388 \pm 800$ µg/L, respectively). However, iGH concentrations were statistically higher ($P < 0.01$) in women ($0.67 \pm 0.19$ µg/L) vs. men ($0.15 \pm 0.08$ µg/L). For the IGF-I system, women had a 20% higher mean concentration of total IGF-I ($P = 0.03$), with no differences between free IGF-1 and IGFBP-2. For other IGF binding proteins, women had 27% higher concentrations of ALS ($P < 0.01$), and 11.5% higher concentrations of IGFBP-3 ($P = 0.01$) when compared to the men.

### 3.3. Bone biomarkers

Measurements for select bone biomarkers are presented in Table 3. There was no significant between-group difference for osteocalcin, intact PTH, and total PTH. The only significant between-group differences occurred for NTx and CTX. The concentration of NTx was 35% higher for the males ($P \leqslant 0.05$), whereas, CTX was 39% higher for the females ($P \leqslant 0.05$).

### 3.4. Correlation analysis

Table 4 displays the correlation matrix between the IGF-I/GH system and bone measures for both men and women. The only significant correlation for men between GH and bone measurements was a significant association of iGH and cortical thickness ($r = 0.42$). For women only, ALS was positively associated with DXA-assessed spine ($r = 0.40$) and femoral neck BMD ($r = 0.42$), and pQCT-assessed tibial BMD ($r = 0.47$) and cortical thickness ($r = 0.40$). Similarly, IGFBP-3 was positively associated with DXA-assessed spine ($r = 0.42$) and femoral neck BMD ($r = 0.47$), and for pQCT-assessed calcaneal BMD ($r = 0.43$). The only significant relationship between bone biomarkers and bone measurements was observed in men, between osteocalcin

Table 1
DXA and pQCT assessed bone health variables for women and men

| Parameter | Women ($n = 39$) | Men ($n = 41$) | Women value as a % of the Men value |
|---|---|---|---|
| Spine BMD | $1.31 \pm 0.02^*$ | $1.22 \pm 0.02$ | 107.5 |
| Total hip BMD | $1.17 \pm 0.02^*$ | $1.23 \pm 0.03$ | 94.9 |
| Femoral neck BMD | $1.17 \pm 0.02^*$ | $1.23 \pm 0.03$ | 95.1 |
| Calcaneal BMD | $0.63 \pm 0.02^*$ | $0.68 \pm 0.01$ | 92.9 |
| Tibial BMD | $792.7 \pm 17.1$ | $783.0 \pm 16.6$ | 101.2 |
| Tibial periosteal circumference | $72.5 \pm 0.9^*$ | $77.2 \pm 0.9$ | 93.9 |
| Cortical thickness | $3.9 \pm 0.1$ | $4.3 \pm 0.1$ | 90.7 |

Absolute values and values adjusted for differences in body mass are provided. Values are adjusted means $\pm$ SEM co-varied for body weight between males and females.
$^*$ $P \leqslant 0.05$ vs. Men.

Table 2
Mean concentrations for the GH/IGF-I system in women and men

| Biomarker | Women ($n = 39$) | Men ($n = 41$) |
|---|---|---|
| Bioassayable GH (µg/L) | $4282 \pm 643$ (750–17145) | $5388 \pm 800$ (750–17327) |
| Immunoreactive GH (µg/L) | $0.67 \pm 0.19$ (0.03–5.20)$^*$ | $0.15 \pm 0.08$ (0.03–3.23) |
| Total IGF-I (µg/L) | $279.1 \pm 15.2$ (80.6–503.8)$^*$ | $232.5 \pm 14.8$ (22.5–431.1) |
| Free IGF-I (µg/L) | $1.2 \pm 0.1$ (0.4–3.4) | $1.3 \pm 0.1$ (0.3–3.2) |
| ALS (µg/mL) | $18.1 \pm 0.6$ (8.2–28.6)$^*$ | $14.2 \pm 0.6$ (6.8–20.1) |
| IGFBP-2 (µg/L) | $362.4 \pm 24.0$ (180.4–910.2) | $344.0 \pm 22.8$ (160.8–789.2) |
| IGFBP-3 (µg/L) | $4052.7 \pm 119.3$ (2250.9–5707.7)$^*$ | $3636.1 \pm 116.4$ (1433.7–4704.4) |

Values are means $\pm$ SEM (range of concentrations included in parentheses).
$^*$ $P \leqslant 0.05$ vs. Men.

Table 3
Mean concentrations for bone biomarkers in women and men

| Bone turnover biomarkers | Women ($n = 36$) | Men ($n = 41$) |
|---|---|---|
| Osteocalcin (µg/L) | 11.6 ± 0.7 (4.7–32.0) | 13.5 ± 0.7 (4.1–21.6) |
| Intact PTH (pg/mL) | 48.9 ± 3.0 (102.5–80.1) | 47.3 ± 2.9 (25.2–99.0) |
| PTH (pg/mL) | 31.8 ± 2.0 (10.0–54.0) | 33.7 ± 2.6 (4.0–73.0) |
| NTx (nM) | 12.3 ± 0.6 (7.3–19.5)[*] | 19.1 ± 1.2 (8.3–49.7) |
| CTX (pg/mL) | 414.1 ± 26.5 (130.7–761.3)[*] | 680.6 ± 57.0 (270.6–2195.0) |

Values are means ± SEM (range of concentrations included in parentheses).
[*] $P \leqslant 0.05$ vs. Men.

Table 4
Correlation matrix between GH/IGF-I system concentrations and bone health measures for women and men

| GH/IGF-I system | DXA | | | | pQCT | | |
|---|---|---|---|---|---|---|---|
| | Spine BMD | Total Hip BMD | Femoral Neck BMD | Calcaneal BMD | Tibial BMD | Tibial Periosteal Circumference | Cortical Thickness |
| **Bioassayable GH** | | | | | | | |
| Women | −0.20 | 0.03 | −0.17 | −0.16 | −0.07 | 0.16 | −0.04 |
| Men | 0.08 | −0.06 | 0.14 | 0.05 | 0.18 | −0.27 | −0.13 |
| **Immunoreactive GH** | | | | | | | |
| Women | −0.02 | −0.14 | −0.18 | 0.02 | −0.09 | −0.04 | −0.12 |
| Men | 0.10 | 0.21 | 0.08 | −0.04 | 0.30 | −0.02 | 0.42[*] |
| **Total IGF-I** | | | | | | | |
| Women | 0.13 | 0.33 | 0.25 | −0.04 | 0.14 | 0.11 | 0.24 |
| Men | 0.22 | 0.21 | 0.21 | 0.13 | 0.00 | 0.04 | 0.00 |
| **Free IGF-I** | | | | | | | |
| Women | −0.24 | 0.25 | −0.06 | −0.02 | 0.01 | −0.02 | −0.01 |
| Men | 0.21 | 0.22 | 0.28 | −0.05 | 0.28 | −0.16 | 0.18 |
| **ALS** | | | | | | | |
| Women | 0.40[*] | 0.25 | 0.42[*] | 0.33 | 0.47[*] | −0.29 | 0.40[*] |
| Men | 0.06 | 0.09 | 0.07 | 0.03 | 0.06 | 0.00 | 0.02 |
| **IGFBP-2** | | | | | | | |
| Women | −0.22 | −0.30 | −0.29 | −0.14 | −0.20 | 0.25 | −0.06 |
| Men | 0.01 | 0.07 | −0.01 | 0.15 | 0.01 | 0.34 | 0.37 |
| **IGFBP-3** | | | | | | | |
| Women | 0.42[*] | 0.28 | 0.47[*] | 0.43[*] | 0.24 | 0.09 | 0.33 |
| Men | −0.02 | −0.05 | 0.01 | −0.08 | −0.36 | 0.31 | −0.09 |

Values reported are Pearson's product moment correlation ($r$).
[*] Significant correlation at $P \leqslant 0.05$.

and tibial periosteal circumference ($r = 0.39$). There were no significant correlations between any bone biomarkers and measurements of bone parameters in women.

## 4. Discussion

The GH/IGF-I axis is a dynamic regulator of tissue growth, particularly with respect to post-natal skeletal development. The current study tested the hypothesis that *in vivo* growth hormone bioactivity (bioGH) and downstream mediators of GH, such as IGF-I and its associated binding proteins, would be associated with *in vivo* DXA and pQCT measures of bone health in

men and women. Although the results of this study demonstrated that bioGH was not significantly associated with BMD in either men or women, IGF-I associated binding proteins (IGFBP-3 and ALS) emerged as significant correlates of BMD exclusively in women.

Contrary to our original hypothesis, bioGH was not significantly associated with any DXA or pQCT-derived measure of bone health in either men or women. The rationale for this hypothesis stemmed from the concept that iGH (GH concentrations determined by immunoassay) represents only an incomplete picture of GH availability, and that a quantifiable measure of the biological activity of GH perhaps would be more likely to better reflect *in vivo* bone measures in humans. bioGH has been the subject of recent investigations involving

exercise [9] and microgravity paradigms [14], although none of these paradigms involved bone-specific outcomes. These existing studies have collectively demonstrated, (1) a dichotomy exists in terms of the relative "responsiveness" of iGH vs. bioGH [9] and that (2) bioGH is influenced by afferent neuromuscular activity [15,16]. For example, Kraemer et al. [9] recently demonstrated that acute resistance exercise elicits an increase in iGH, but not bioGH, while chronic resistance exercise training elicits an increase in bioGH, but not iGH. A partial explanation for these discrepancies between acute and chronic exercise may lie in the degree to which growth hormone exhibits a great deal of molecular heterogeneity [10,17]. Indeed, higher molecular weight forms of GH (>60 kDa) have low immunodetectability but have high bioactivity, and it has been suggested that exercise may preferentially elicit increases in higher molecular weight forms of GH (e.g., dimeric forms) and possibly prolonging GH half-life as evidenced by in increase in immunoassayable GH following chemical reduction *via* glutathione [8]. Further, both bed rest and spaceflight (conditions both associated with bone loss) have been shown to diminish the responsiveness of bioGH, but not iGH, to exercise [5,14]. These data point out the complexity with regard to GH physiology and illustrate the importance of framing conclusions on GH within both methodological and experimental paradigms.

To our knowledge, this the first study to directly compare bioGH concentrations between sexes. In our group of age-matched men and women, it was of interest to note that, (a) statistically similar bioGH concentrations were observed, and (b) that the bioGH concentrations in the current investigation were similar to bioGH concentrations observed in the previous studies [5, 8, 9, 14]. The lack of any significant association between bioGH and BMD may perhaps reflect a greater relative importance of GH pulsatility in determining somatogenic actions regulated by GH. Other possible explanations for the lack of an association between bioGH and the bone parameters measured in the current study may include the fact that bioGH is only one factor of many impacting peak bone mass [13], only one timepoint samples were analyzed, or that the subjects in the study were all self-selected, physically fit military cadets. As such, their BMD measures may represent a narrower spectrum well above age-matched normative data, or that the BMD measures in this study assessed mature fused bones and the bioassay measured developing bone at the growth plate. Other GH bioassays relevant to bone exist, such as the *in vitro* bioassays utilizing the human osteoblastic Saos-2 cell line [6], and should be considered in future studies.

Consistent with an earlier report from our laboratory that demonstrated a gender-specific relationship between the IGF-I system and bone mineral content in age-matched (29 years) men and women, this study revealed that IGFBP-3 and the ALS were associated with BMD in women, but not men [11]. This apparent gender specificity in the association between the IGF-I system and bone health, while currently unexplained, is consistent with other reports in the literature [1, 3]. Further, our data suggest that, at least in women, systemic bioactive GH and systemic IGF-I may have disparate influences on bone turnover [18].

The IGF-I system is thought to mediate many of the downstream effects of GH, and it is now even recognized that IGFBPs exert their own independent effects [19]. While our previous report demonstrated that total IGF-I was associated with bone mineral content in women [11], neither total or free IGF-I were significantly associated with BMD in the current study. Similarly to IGF-I, the associated binding proteins, IGFBP-3 and ALS, are under the direct regulation of GH as well. The ternary complex consisting of IGF-I, IGFBP-3, and the ALS sequester the majority of circulating IGF-I (75–90%) and serves to prolong the half-life of IGF-I. While it is possible that local IGF-I may exert stronger control over local bone turnover and metabolism than systemic IGF-I, the seminal work of Yakar et al. [4, 20] has demonstrated the importance of both circulating IGF-I and the ternary complex for bone remodeling *via* the liver IGF-I deficient mouse and the ALS knockout mouse. The current significant correlation between IGFBP-3 and ALS and bone properties contribute to an expanding body of evidence illustrating that IGF binding proteins also possess independent actions/roles.

In summary, this study has evaluated the relationship between circulating components of the GH/IGF-I system and bone health, with particular regard to bioactive GH and IGFBPs. Contrary to the original hypothesis, *in vivo* bioGH was not correlated with any measure of bone health in either men or women. Furthermore, a gender-specific (women only) relationship was observed for IGFBP-3 and ALS in certain parameters of DXA- and pQCT-assessed bone health, which further supports the binding proteins' independent growth effects on BMD. Future studies are required to examine the mechanisms underlying (1) the stronger relationship for IGFBPs vs. IGF-I in terms of bone remodeling and (2) the apparent gender-specific relationship between the IGF-I system and bone remodeling.

## 5. Disclaimer

prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32 CFR Part 219. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on the use of volunteers in research. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

## Acknowledgement

## References

[1] E. Barrett-Connor, D. Goodman-Gruen, Gender differences in insulin-like growth factor and bone mineral density association in old age: the Rancho Bernardo Study, J. Bone Miner. Res. 8 (1998) 1343–1349.

[2] F.S. Greenspan, C.H. Li, M.E. Simpson, H.M. Evans, Bioassay of hypophyseal growth hormone; the tibia test, Endocrinology 5 (1949) 455–463, Illust.

[3] J.A. Langlois, C.J. Rosen, M. Visser, et al., Association between insulin-like growth factor I and bone mineral density in older women and men: the Framingham Heart Study, J. Clin. Endocr. Metab. 12 (1998) 4257–4262.

[4] S. Yakar, M.L. Bouxsein, E. Canalis, et al., The ternary IGF complex influences postnatal bone acquisition and the skeletal response to intermittent parathyroid hormone, J. Endocrinol. 2 (2006) 289–299.

[5] G.E. McCall, C. Goulet, R.E. Grindeland, J.A. Hodgson, A.J. Bigbee, V.R. Edgerton, Bed rest suppresses bioassayable growth hormone release in response to muscle activity, J. Appl. Physiol. 6 (1997) 2086–2090.

[6] P.G. Ziros, T. Georgakopoulos, I. Habeos, E.K. Basdra, A.G. Papavassiliou, Growth hormone attenuates the transcriptional activity of Runx2 by facilitating its physical association with Stat3beta, J. Bone. Miner. Res. 11 (2004) 1892–1904.

[7] C. Ohlsson, B.A. Bengtsson, O.G. Isaksson, T.T. Andreassen, M.C. Slootweg, Growth hormone and bone, Endocr. Rev. 1 (1998) 55–79.

[8] W.C. Hymer, W.J. Kraemer, B.C. Nindl, et al., Characteristics of circulating growth hormone in women after acute heavy resistance exercise, Am. J. Physiol. Endocr. Metab. 4 (2001) E878–E887.

[9] W.J. Kraemer, B.C. Nindl, J.O. Marx, et al., Chronic resistance training in women potentiates growth hormone *in vivo* bioactivity: characterization of molecular mass variants, Am. J. Physiol. Endocr. Metab. 6 (2006) E1177–E1187.

[10] B.C. Nindl, W.J. Kraemer, J.O. Marx, A.P. Tuckow, W.C. Hymer, Growth hormone molecular heterogeneity and exercise, Exercise Sport. Sci. Rev. 4 (2003) 161–166.

[11] B.C. Nindl, C.R. Scoville, K.M. Sheehan, C.D. Leone, R.P. Mello, Gender differences in regional body composition and somatotrophic influences of IGF-I and leptin, J. Appl. Physiol. 4 (2002) 1611–1618.

[12] J. Ruffing, F. Cosman, M. Zion, et al., Determinants of bone mass and bone size in a large cohort of physically active young adult men, Nutr. Metab. (2006) 14.

[13] J.A. Ruffing, J.W. Nieves, M. Zion, et al., The influence of life style, menstrual function and oral contraceptive use on bone mass and size in female military cadets, Nutr. Metab. (2007) 17.

[14] G.E. McCall, C. Goulet, R.R. Roy, et al., Spaceflight suppresses exercise-induced release of bioassayable growth hormone, J. Appl. Physiol. 3 (1999) 1207–1212.

[15] K.L. Gosselink, R.E. Grindeland, R.R. Roy, et al., Skeletal muscle afferent regulation of bioassayable growth hormone in the rat pituitary, J. Appl. Physiol. 4 (1998) 1425–1430.

[16] K.L. Gosselink, R.R. Roy, H. Zhong, R.E. Grindeland, A.J. Bigbee, V.R. Edgerton, Vibration-induced activation of muscle afferents modulates bioassayable growth hormone release, J. Appl. Physiol. 6 (2004) 2097–2102.

[17] G. Baumann, Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins, Endocr. Rev. 4 (1991) 424–449.

[18] T. Ueland, Bone metabolism in relation to alterations in systemic growth hormone, Growth Horm. IGF Res. 6 (2004) 404–417.

[19] S. Mohan, D.J. Baylink, IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms, J. Endocrinol. 1 (2002) 19–31.

[20] S. Yakar, C.J. Rosen, W.G. Beamer, et al., Circulating levels of IGF-1 directly regulate bone growth and density, J. Clin. Invest. 6 (2002) 771–781.